

Mode of Action of Psoralens, Benzofurans, Acridinons, and Coumarins on the Ionic Currents in Intact Myelinated Nerve Fibres and its Significance in Demyelinating Diseases

K. H. BOHUSLAVIZKI¹, W. HÄNSEL², A. KNEIP¹, E. KOPPENHÖFER¹,
E. NIEMÖLLER² and K. SANMANN²

¹ Institute of Physiology, University of Kiel,
Olshausenstr. 40, 24098 Kiel, Germany

² Institute of Pharmacy, University of Kiel,
Gutenbergstr. 76–78, 24118 Kiel, Germany

Abstract. The actions of psoralens, benzofurans, acridinons and coumarins on the ionic currents in intact myelinated nerve fibres were investigated. All 6 substances blocked the potassium currents in a time-dependent manner, producing so-called K⁺ transients. Only 5-methoxypsoralen is a largely selective blocker of predominantly the axolemmal potassium channels, which is the characteristic required by our previously proposed working hypothesis for the mechanism of potassium-channel blockers in demyelinating diseases, in particular multiple sclerosis. If the observed K⁺ transients were to arise by blocking of the potassium channels of the Schwann cell, that is, by the periaxonal accumulation of K⁺ and a resulting collapse of the electromotive driving force for potassium-ions, according to a modified version of our previous hypothesis the other substances tested could also have a beneficial effect on the impaired impulse conduction in demyelinated axons. In this case a large number of new potential drugs would be available for the symptomatic therapy of MS.

Key words: Node of Ranvier — K⁺ transients — K⁺-channel blockers — Psoralens — Benzofurans — Acridinons — Coumarins — Demyelinating diseases

Introduction

Selective pharmacological blockers of either sodium or potassium ionic currents have been considered useful tools for a closer understanding of potential dependent

Correspondence to: Prof. Dr. med. E. Koppenhöfer, Institute of Physiology, University of Kiel, Olshausenstr. 40, 24098 Kiel, Germany

ionic channels in the intact nerve fibre. Apart from this, potassium channel blockers like 4-aminopyridine (see e.g. Stefoski et al. 1991; Van Diemen et al. 1992, 1993a,b) and, in particular, psoralens (Bohuslavizki et al. 1993a,b) and related substances like acridinons (Bohuslavizki et al. 1993c) and coumarins (Bohuslavizki et al. 1993d, 1994) have received much interest as a symptomatic therapy in multiple sclerosis (MS) and a variety of peripheral neuropathies. The reason is that many potassium channels are abnormally exposed in demyelinated nerve fibres (Chiu and Ritchie 1982; Grissmer 1986; Chiu and Schwarz 1987; Jonas et al. 1989), which render nerve function difficult either by an increase or by a decrease in membrane potential (Waxman 1987; Bautz et al. 1989; Bohuslavizki et al. 1992). Therefore, according to common belief, potassium channel blocking agents are basically suited to improve functional deficits seen in MS and related diseases.

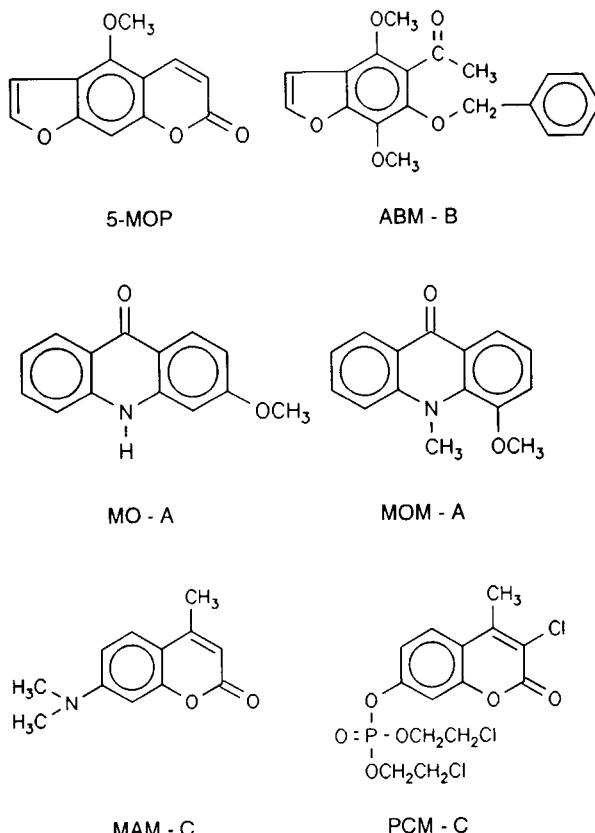


Figure 1. Molecular structures of the substances tested. 5-MOP: 5-methoxypsoralen. ABM-B: 5-acetyl-6-benzyl-4,7-dimethoxybenzofuran. MOM-A: 4-methoxy-N-methyl-acridin-9-on. MO-A: 3-methoxy-acridin-9-on. MAM-C: 7-dimethylamino-4-methylcoumarin. PCM-C: phosphoric acid-bis-(2-chlor-ethyl)-(3-chlor-4-methyl-7-coumarin).

The aim of the present study was to investigate the mode of action of psoralens, benzofurans, acridinons and coumarins (Fig. 1) in demyelinating diseases (Bautz et al. 1989; Bohuslavizki et al. 1992) by *in vitro* experiments on intact myelinated axons in the light of the Hodgkin-Huxley-Frankenhaeuser formalism (Hodgkin and Huxley 1952; Frankenhaeuser and Huxley 1964).

Materials and Methods

Chemicals and solutions

7-dimethylamino-4-methylcoumarin (MAM-C), phosphoric acid-bis-(2-chlorethyl)-(3-chlor-4-methyl-7-coumarin) (PCM-C) and 5-methoxysoralen (5-MOP) were purchased from Aldrich Chemie (Steinheim, Germany); the benzofuran and the acridinons were synthesized. Stock solutions (2 mmol/l) of all substances were first prepared with dimethylsulfoxide (DMSO; Fluka AG, Buchs, Switzerland). For the experiments, these were diluted with Ringer solution so that in the final test solutions the concentration of the test substance was uniformly 100 µmol/l and that of DMSO was 0.7 mol/l. Data collection was begun as soon as the measured effect of the test substance had stabilized at a constant level. This took 5 min in all cases at the most. The subsequent washing-out phase was as long as necessary for complete reversibility, between 3 and 15 min.

The normal bathing medium was either Ringer solution (in mmol/l): NaCl 107.0; KCl 2.5; CaCl₂ 2.0; N,N-bis(hydroxyethyl)-2-amino-ethanesulfonic acid/NaOH buffer (BES) 5.0 or a K⁺-rich solution (in mmol/l): KCl 108.0; NaCl 5.0; CaCl₂ 2.0; BES 5.0. The solution for use as artificial intracellular fluid contained (in mmol/l): KCl 108.0; NaCl 5.0; BES 5.0. The pH of all solutions was 7.2 ± 0.1; the temperature during the experiments was 10.0 ± 0.5 °C.

Synthesis

5-acetyl-6-benzyloxy-4,7-dimethoxybenzofuran (ABM-B) was synthesized from Khellinon (Lancaster Synthesis, Morecambe, England) and benzylbromide with potassium carbonate suspended in acetone (Abu-Shady and Soine 1953).

3-methoxy-acridin-9-on (MO-A) was synthesized from 2-chlorobenzoic acid and 3-methoxyaniline (both from Merck, Darmstadt, Germany). N-(3-methoxyphenyl)-antranilic acid was prepared according to the Ullmann reaction (Ullmann 1907), modified by using N,N-dimethyl formamide as a solvent and a mixture of copper and tin powder as catalysts. Treatment of N-(3-methoxyphenyl)-antranilic acid with polyphosphoric acid led to a mixture of stereoisomers of which MO-A was isolated by column chromatography.

4-methoxy-N-methyl-acridin-9-on (MOM-A) was synthesized by methylation of 4-methoxy-acridin-9-on (Aldrich, Steinheim, Germany) under phase-transfer conditions (Galy et al. 1979) in which tetra-N-butylammonium bromide was used as phase-transfer agent.

Statistics

Results are given as medians (Sachs 1984). The significance of effects was tested on the $\alpha = 0.05$ level by the two-sided statistical sign-test (Dixon and Mood 1946).

Preparation and experimental setup

The experiments were done on isolated intact myelinated nerve fibres (diameter: 25 µm, range: 22 to 27 µm; $n = 14$) from the sciatic nerve of the toad *Xenopus laevis*. The

dissection procedure chosen (Koppenhöfer et al. 1987) yielded axons which were obviously superior to those used previously. Thus, experiments several hours in duration were possible, so that the number of animals to be sacrificed was considerably reduced. Measurements of membrane currents were carried out by means of an optimized two-loop potential clamp system which minimizes errors in measurement nearly to the limit to which they can be pushed at present (Bethge et al. 1991).

Measuring conditions and calibrations

All membrane current measurements were done under current-proportional positive feedback (Koppenhöfer and Schumann 1979; Wiese and Koppenhöfer 1988; Bethge et al. 1991). The problem of the suitable amount of feedback and further technical details will be the subject of a forthcoming paper.

The holding potential was adjusted under the assumption of a steady-state value of the sodium inactivation variable $h_\infty = 0.8$. For current-voltage relations various positive test pulses, V , 50 ms in duration were preceded by negative prepulses of amplitude and duration such that at the beginning of test pulses h_∞ was unity. For sodium inactivation curves the potential dependence of h_∞ was measured (Frankenhaeuser 1959).

Specific currents were calculated from membrane current records and from the fibre dimensions according to the passive standard data for myelinated nerve fibres (Stämpfli and Hille 1976).

Data processing

To calculate the membrane permeabilities P_{Na} and P_K from the underlying ionic currents I_{Na} and I_K , the constant-field concept was applied (Dodge and Frankenhaeuser 1959; Frankenhaeuser 1962a):

$$P_{Na} = I_{Na} \cdot \frac{RT}{F^2(V + E_R)[Na]_0} \cdot \frac{\exp\{(V + E_R)F/RT\} - 1}{\exp\{(V - V_{Na})F/RT\} - 1} \quad (1)$$

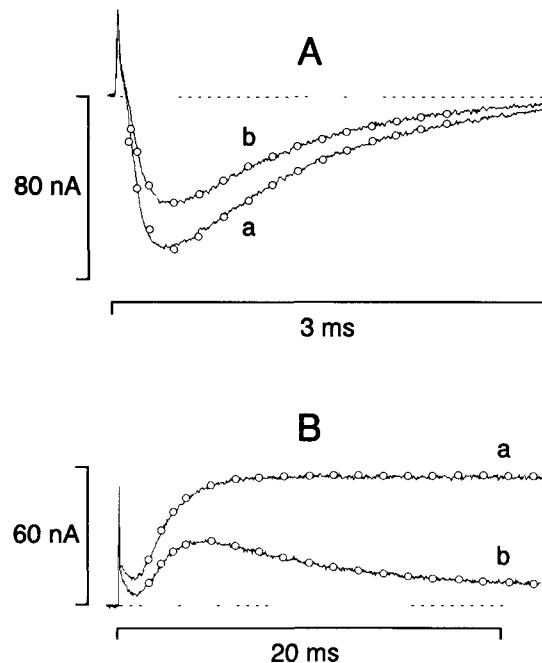
$$P_K = I_K \cdot \frac{RT}{F^2(V + E_R)[K]_0} \cdot \frac{\exp\{(V + E_R)F/RT\} - 1}{\exp\{(V - V_K)F/RT\} - 1}, \quad (2)$$

where V is the change in membrane potential with respect to the resting potential E_R and V_{Na} and V_K are the electrochemical equilibrium potentials for sodium and potassium ions, respectively; $[Na]_0$, $[K]_0$, R , T and F have their usual meanings. In the case of P_{Na} this procedure is justified for the physiologically relevant potential range (Albers et al. 1989; Neumann 1991) and for P_K it is evidently a useful approximation (Frankenhaeuser 1962a).

The permeability constants \bar{P}_{Na} and \bar{P}_K were found by computer-assisted calculation of P'_{Na} and P'_K , respectively, from the time courses of the membrane-current records by least-square fitting (Fig. 2). In determining P'_{Na} the disturbing influences of the capacitive current at the beginning of the record and, especially, of the superimposed potassium current at its end were reduced by neglecting the first 25–30% of the peak sodium current in each case (A). In determining P'_K it was only at the beginning of the record that the first 30–35% of the total amplitude of the steady-state potassium current had to be neglected, because of the superimposed sodium currents (B).

We found V_{Na} from the sodium current-voltage curves; the potassium equilibrium potential V_K was taken to be –22 mV (Frankenhaeuser and Huxley 1964) and for the

Figure 2. Curves fitted to early inward currents (*A*) with equation 3 and to late outward currents (*B*) with equations 4 and 5. a: Ringer solution; b: test solution (PCM-C); open circles: calculated data, $r_{nl} > 0.996$; test-pulse amplitude: $V = 70$ mV (*A*) or 150 mV (*B*); dotted line: zero membrane current. The records were taken from the corresponding measurement series represented in Fig. 4.



resting potential an average value of $E_R = -70$ mV (Dodge and Frankenhaeuser 1959) was assumed. The test-pulse amplitudes for measuring P'_{Na} were limited to the range $V = 40$ to 110 mV and those for P'_K were in the range $V = 80$ to 160 mV. Hence it was possible to use the simplified equations

$$P_{Na} = P'_{Na} \cdot \{1 - \exp(-t/\tau_m)\}^a \cdot \exp(-t/\tau_h) \quad (3)$$

and

$$P_K = P'_K \cdot \{1 - \exp(-t/\tau_n)\}^b \quad (4)$$

to fit the curves (Frankenhaeuser 1960). Here τ_m , τ_n , and τ_h are the time constants of activation and inactivation of the respective permeabilities, P'_{Na} and P'_K are potential-dependent variables, and a and b are empirical exponents of the associated activation terms.

For the potassium currents recorded in test solutions, equation (4) was expanded by a corresponding inactivation term (Frankenhaeuser 1963):

$$P_K = P'_K \cdot \{1 - \exp(-t/\tau_n)\}^b \cdot \{k_\infty + (1 - k_\infty)\} \cdot \exp(-t/\tau_k), \quad (5)$$

where τ_k is the time constant of inactivation and k_∞ is the steady-state value of the inactivation variable k of the potassium system.

As a measure of the quality of the curve-fitting we used the nonlinear regression coefficient r_{nl} (Sachs 1984). It was at least 0.995.

Results

DMSO effects. In control experiments DMSO, at the concentration used here (0.7 mol/l), proved to be inert with respect to the sodium inactivation curve. The potassium currents I_K , however, were reversibly reduced by DMSO in the potential range studied by 19% (range: 14 to 22%; $n = 14$) regardless of the potential, while there was a reversible increase in the sodium currents I_{Na} by 4% (range: -2 to 10%; $n = 14$). Therefore all the I_K and I_{Na} values measured in each test solution were processed to eliminate the superimposed DMSO effect before further evaluation.

Sodium inactivation curves. The data on potential dependence of the parameter h_∞ of sodium inactivation were fitted by the empirical equation

$$h_\infty = \frac{1}{1 + \exp [(V - V_h)/k_h]} \quad (6)$$

(Frankenhaeuser 1959). The various test substances shifted the inactivation curve in the negative direction to various degrees (Table 1). The smallest leftward shifts ($-\Delta V_h$) were caused by 5-MOP and MAM-C. The slope of the curves (k_h) was not affected by any of the substances tested here.

Table 1. Influence of the substances tested on sodium inactivation and on the permeability constants \bar{P}_{Na} and \bar{P}_K . $-\Delta \tilde{V}_h$: leftward shift of the inactivation curve in mV, with reference to the normal values in Ringer solution; $\Delta \tilde{P}_{Na}$, $\Delta \tilde{P}_K$: changes in the permeability constants as a percentage of the normal values of each in Ringer solution. Median values from 10 series of measurements on a total of 14 axons in each case.

	$-\Delta \tilde{V}_h$	$\Delta \tilde{P}_{Na}$	$\Delta \tilde{P}_K$
	[mV]	[%]	[%]
5-MOP	3	-17.8	-34.2
ABM-B	14	-43.4	1.7
MO-A	7	-16.7	-4.5
MOM-A	10	-20.6	-10.7
MAM-C	4	0.0	2.9
PCM-C	7	-26.5	21.7

Time course of current records. The time course of the sodium currents elicited by depolarizing pulses of various amplitudes was unaffected by the substances used in these experiments (Fig. 2A). The potassium currents, however, acquired the phasic time course (Figs. 2B and 3) previously described for benzofurans and acridinones (Bohuslavizki et al. 1993 a,c,d; 1994). Whereas normally (curves a)

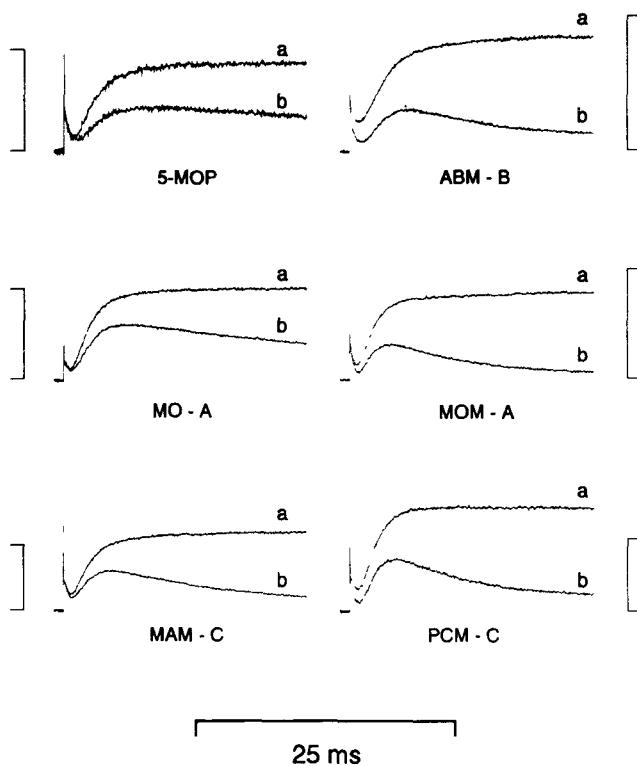


Figure 3. Membrane-current records under the influence of the indicated test substances (b) in comparison to records in normal Ringer solution (a). Test-pulse amplitude: $V = 150$ mV. The vertical bars indicate 40 nA. The records were taken from the corresponding measurement series represented in Fig. 4.

the potassium currents rise monotonically to a stationary final value, under the influence of the substances tested here they first reach a relative maximum within a few milliseconds and then approach a lower steady-state value (b). These so-called K⁺ transients are noticeably less well-defined under 5-MOP and also MO-A than under the other test substances.

Current-voltage relations. The potential dependence of the peak sodium currents (peak I_{Na}), the steady-state level of the potassium currents (I_{Kss}) and the transient maxima of the potassium currents under the influence of the test substances (peak I_K) is illustrated in the form of current-voltage curves (Fig. 4). The action of the test substances on peak I_{Na} (triangles) consisted in a potential-independent reduction by an amount depending on the substance: only 5-MOP failed to affect peak I_{Na} . The observed blocking of the potassium currents was also potential-

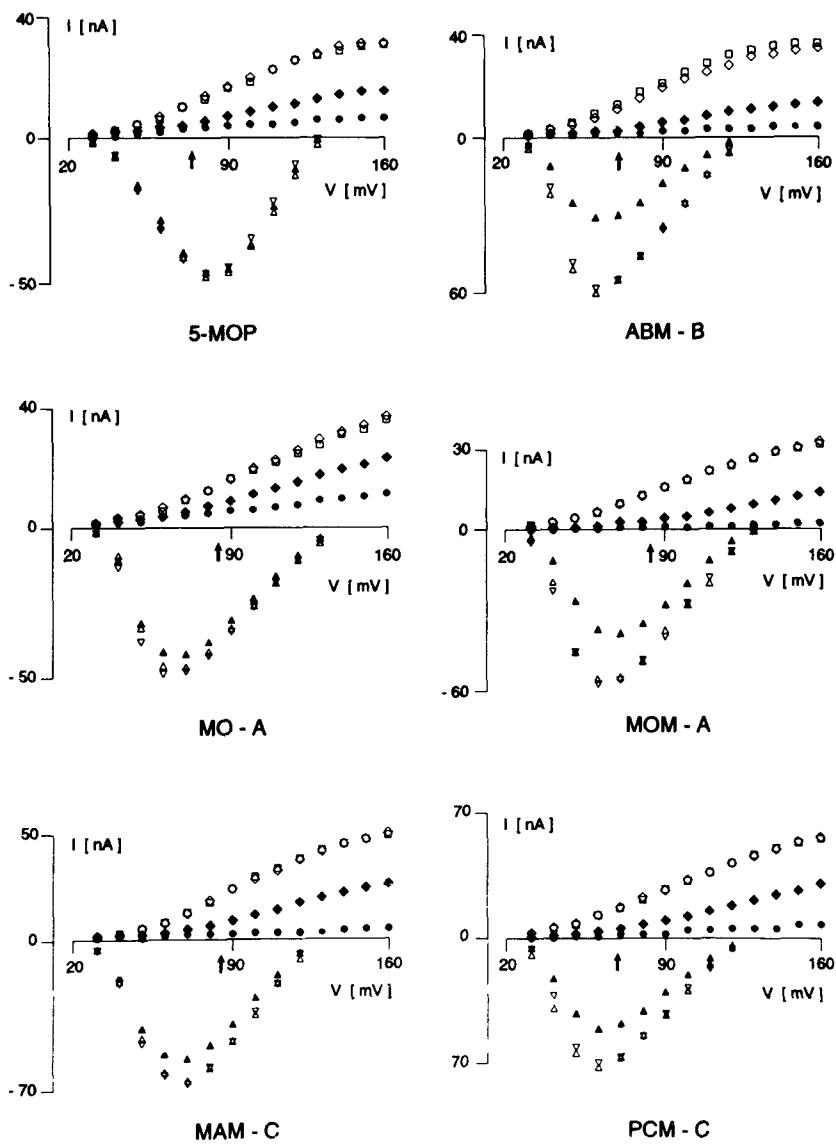


Figure 4. Current-voltage relations of steady-state potassium currents (squares and diamonds) potassium transients (filled circles) and peak sodium currents (triangles) before and after application of test solutions (open symbols). Test solutions (filled symbols) 100 $\mu\text{mol/l}$ throughout abscissae test pulse amplitude V in mV ordinates corresponding ionic currents, I in nA. Arrows denote zero membrane potential ($E = 0$ mV) assuming that the potential measured in pool A *versus* ground indicates the absolute membrane potential. Note that the minimal DMSO induced changes of current amplitudes are superimposed. Plots are from 6 individual tests.

Table 2. Influence of the test substances on the time constants of potassium activation τ_n and of potassium inactivation τ_k as well as on the parameter $k_\infty \cdot -\Delta \tilde{\tau}_n$: decrease of $\tilde{\tau}_n$ as a percentage of the normal value in Ringer solution in each case. Each value in the Table is the median of 10 measurement series on a total of 14 axons, measured at $V = 90$ or 160 mV.

V [mV]	$-\Delta \tilde{\tau}_n$ [%]		$\tilde{\tau}_k$ [ms]		\tilde{k}_∞	
	90	160	90	160	90	160
5-MOP	47	-26	18	12	0.23	0.19
ABM-B	44	25	8	7	0.06	0.12
MO-A	28	24	25	20	0.41	0.33
MOM-A	62	34	10	9	0.05	0.08
MAM-C	50	17	24	20	0.14	0.16
PCM-C	36	-2	8	7	0.12	0.14

independent, with respect to both the steady-state values I_{Kss} (filled circles) and the transient peak I_K (filled diamonds). Moreover, the blocking of I_{Kss} by 5-MOP and, in particular by MO-A, was clearly less effective than by the other test substances.

Curve fitting. By using equations 3 and 4 to fit curves to the current records, median values were obtained for the time constants of activation of the sodium and potassium permeabilities, τ_m and τ_n , respectively, as well as the exponents a and b , the time constant of sodium inactivation τ_h and the permeabilities P'_{Na} and P'_K . In addition, under the assumption that the solutions tested here bring about potassium inactivation, curve fitting with equation 5 provide median values for the time constant of potassium inactivation τ_k as well as for the variable of potassium inactivation k_∞ .

The fits showed no influence of the test solutions on the time constants for sodium permeability, τ_m and τ_h , or on the exponent a (not tabulated). The time constant for potassium activation τ_n , however, was mostly reduced (Table 2), while the exponent b was about doubled (not tabulated). The time constant of potassium inactivation τ_k and the parameter k_∞ , when measured in the test solutions, were potential-independent to a good approximation within the range of the measurements. Table 2 also shows that the lesser time dependence of the potassium currents recorded under 5-MOP and MO-A, in comparison with the rest of the substances tested (Fig. 3), is caused not by slower but by weaker inactivation; that is, it is the result of a larger steady-state value of the inactivation variable k_∞ .

Permeability-voltage relations. The potential dependence of the median values of

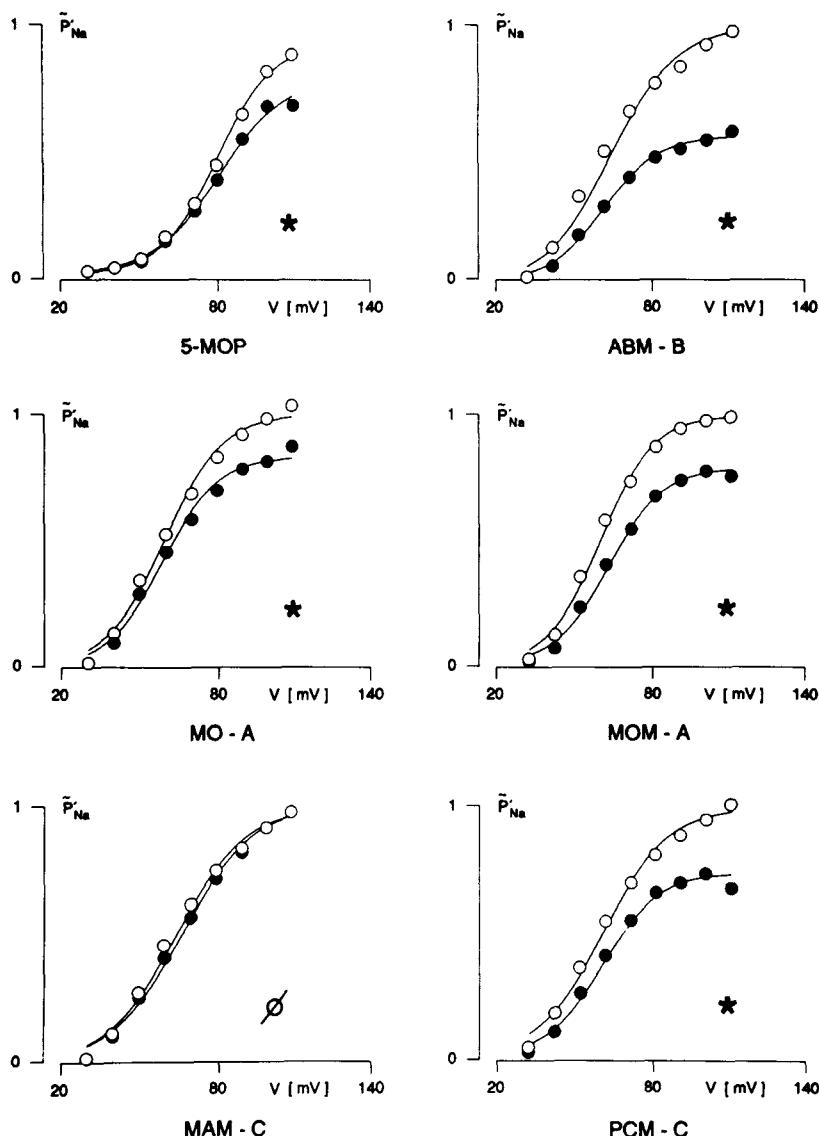


Figure 5. Median sodium permeabilities, \tilde{P}'_{Na} , as calculated for median sodium currents, \tilde{I}'_{Na} , by equations 1 and 3. Abscissae: test pulse amplitude, V , in mV; ordinates: \tilde{P}'_{Na} normalized to the maximum of the continuous curve which was calculated by equation 7 and fitted to the data in Ringer solution before and after application of test solutions (open symbols); dashed line: corresponding curve fitted to the data in the respective test solution (filled symbols). $r_{\text{nt}} > 0.995$ throughout. Data from 10 tests for each drug obtained from a total of 14 axons. *: significant changes; \circ : nonsignificant changes.

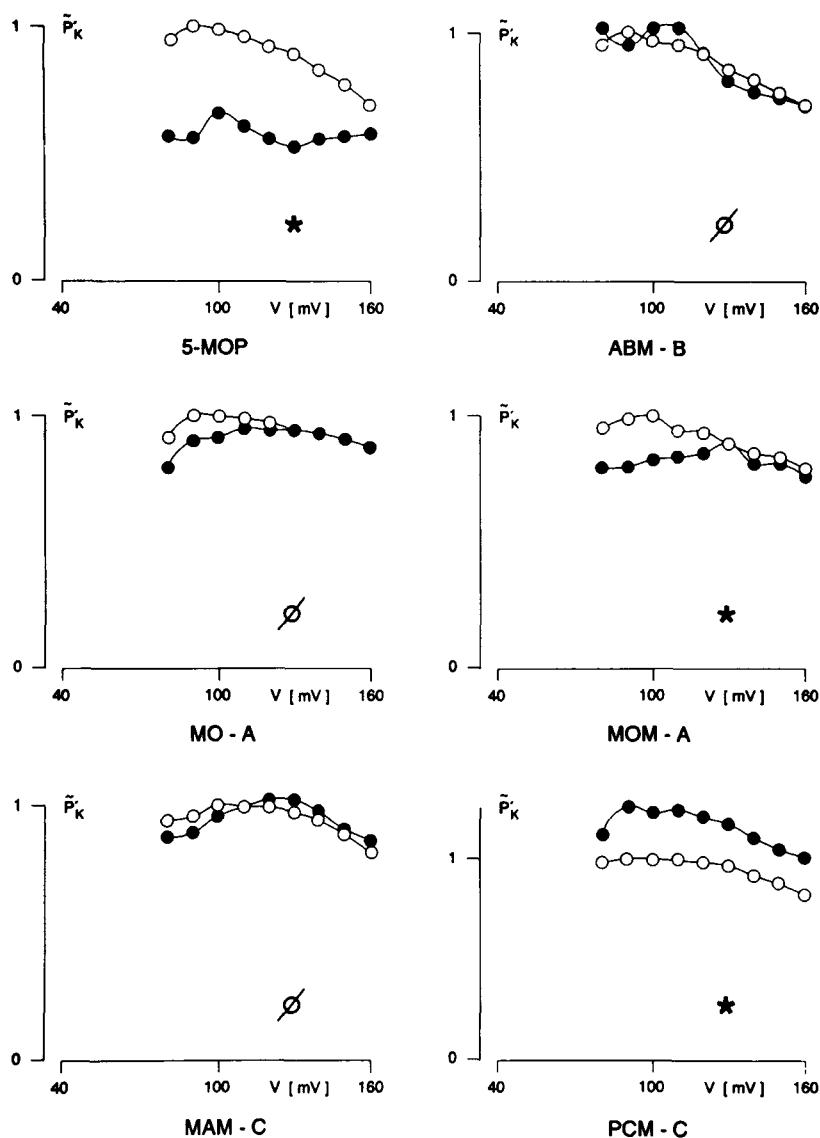


Figure 6. Median potassium permeabilities, \tilde{P}'_K , as calculated for median potassium currents, I'_K , by equations 2, 4 and 5, respectively. Abscissae: test pulse amplitude, V , in mV; ordinates: \tilde{P}'_K normalized to maximum of \tilde{P}'_K in Ringer solution before and after application of test solutions (open symbols); filled symbols: data from the respective test solutions. Measured points were interconnected by spline interpolation (Wiese and Koppenhöfer 1988). Data from 10 tests for each drug obtained from a total of 14 axons. *: significant changes; \circ : nonsignificant changes.

P'_{Na} , \tilde{P}'_{Na} in the various test series is shown in Fig. 5. First the equation

$$P'_{\text{Na}} = \bar{P}_{\text{Na}} \cdot \left[\frac{1}{1 + \exp[(V_P - V)/k_P]} \right]^a \quad (7)$$

(Benoit and Dubois 1987) was fitted to the P'_{Na} values measured in Ringer solution. Here V_P is the position of the inflection point of the curve on the potential axis, k_P is the maximal slope of the curve and a is its exponent. The extrapolated maximum of the calculated curve gave the median permeability constant \hat{P}_{Na} in Ringer solution in each case, which was then used to normalize the values of \tilde{P}'_{Na} found for the associated test solution, again by fitting equation 7. All the test substances except MAM-C reduced the permeability constant \bar{P}_{Na} (Table 1). 5-MOP occupies a special position in that although it does reduce \bar{P}_{Na} it has no effect on sodium permeability at weak positive pulses.

Another salient feature of the 5-MOP graph is that even the reference P'_{Na} curve (in Ringer solution) measured in this test is shifted in the positive direction, by $\Delta V_P \approx 20$ mV, in comparison with the other reference curves in Fig. 5. We ascribe this to the fact that in these experiments, to compensate the influence of the series resistance, a systematically stronger positive feedback was used than for the other substances tested. The slope of the curves, k_p , and their exponent, a , were not affected by the different amount of compensation. The test substances also had no effect on the exponent a of the \tilde{P}'_{Na} curves (nor on their slope V_P); to a good degree of precision a was 1.0 (range: 1.0 to 1.4). Therefore in Fig. 5 the open symbols represent so-called activation curves for sodium permeability in Ringer solution, and hence they are directly comparable to the corresponding curves of Frankenhaeuser (1960), Koppenhöfer and Schmidt (1968) and Albers et al. (1989).

The potential dependence of the median values of P'_K , \tilde{P}'_K in the various test series is shown in Fig. 6. There would have been no point in fitting an equation corresponding to equation 7 to these data, because no sigmoid curve is discernible here, for two reasons: there tends to be a decline in the range of strong depolarizations, and for weak depolarizations no data are available. Therefore the maxima of the \tilde{P}'_K curves in Ringer solution (open symbols) were taken as representing the maximal potassium permeability \bar{P}_K and all the \tilde{P}'_K values to be compared were normalized to this value. The changes in \bar{P}_K brought about by each test substance (filled symbols) are also summarized in Table 1. Neither ABM-B nor MAM-C affected the permeability constant \bar{P}_K . Surprisingly, PCM-C produced a clear increase in \bar{P}_K ; it follows that the low steady-state value in the potassium-current recordings (Fig. 3) must have other causes (see pp. 321ff.). In contrast, the action of 5-MOP differs from that of all the other substances tested here; it produces a considerable reduction of \bar{P}_K , distinctly greater than that effected by the acridinons.

Discussion

Drug effects. The action of the substances studied in these experiments on the potassium currents is characterized by the phasic nature of the components that are not blocked. Similar K⁺ transients can be elicited at the membrane of the nodes of Ranvier by various other substances, such as (intracellularly administered) quaternary ammonium compounds (Armstrong and Hille 1972), capsaicin (Dubois 1982) and crown ether (Århem et al. 1982; Kristbjarnarson and Århem 1985). The presumed cause of such time-dependent potassium currents has been a great acceleration of potassium inactivation, the normal inactivation of potassium permeability being extremely slow (Frankenhaeuser 1963; Schwarz and Vogel 1971). Therefore this was our starting assumption for quantification of the results presented here.

The measurement technique we used allows extensive compensation of the disturbing influence of the so-called series resistance on the sodium currents (p. 312). However, because the potassium channels of the Ranvier node membrane are mostly in the paranode region (Chiu and Ritchie 1980; Brismar 1981; Waxman 1987) and accordingly are influenced by a systematically larger series resistance than the sodium channels, which tend to be centrally located, our refinement of measurement technique could not prevent associated systematic errors in our potassium-current measurements. The ultrastructure of the node of Ranvier in the central nervous system of the cat closely resembles that in the peripheral nervous system of *Xenopus* (M. Rydmark, personal communication); hence it is likely that in our preparation, as in cat neurons, the series resistances of various potassium-channel populations differ from one another (Black et al. 1990). It comes as no surprise, then, that the constant-field concept we have used (Frankenhaeuser 1962a) is not a satisfactory description of the potassium permeability (Fig. 5).

In view of the structural diversity of substances, all of which induce K⁺ transients of similar appearance at the membrane of the node of Ranvier, it is surely wrong to assume that in all cases a single receptor in the pharmacological sense is responsible for extremely accelerated inactivation especially since many actions of substances are brought about without the mediation of receptors (Jedicke et al. 1988). It has long been known that the axolemma includes several types of potassium channels (see, e.g., Safronov et al. 1993) with distinct pharmacological properties (Dubois 1982; Bernardi et al. 1989). At present, therefore, the possibility cannot be ruled out that the K⁺ transients we observed are based on a relatively low sensitivity of a small population of a rapid potassium-channel type to the substances we tested; the observed acceleration of potassium activation (Table 2) supports this interpretation.

It is generally accepted that K⁺ uptake into the Schwann cell overlying the axon cylinder is so effective that under physiological conditions periaxonal K⁺

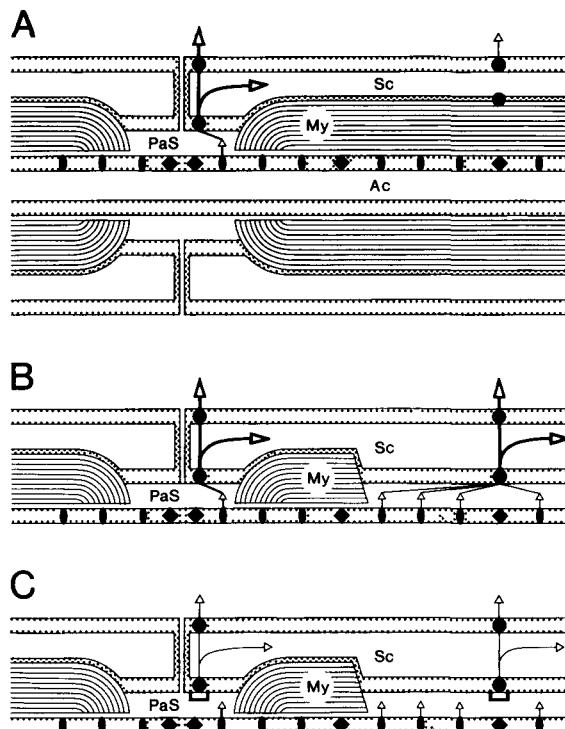


Figure 7 Highly simplified model of the mechanism of action of potassium channel blockers that cause K⁺ transients. Ac axon cylinder My myelin Sc Schwann cell PaS periaxonal space ● potassium channels of the axolemma and the Schwann cell membrane respectively ♦ sodium channels A intact axon B focal demyelination K⁺-uptake by the Schwann cell being normal (bold arrows) C application of potassium channel blockers (□) resulting in decrease of K⁺ uptake (slim arrows). Note that in B and C only half of the fibre is shown

homeostasis applies (Brunder and Lieberman 1988 Hassan and Lieberman 1988 Konishi 1990) On the other hand reductions of potassium currents are probably always (see e.g. Koppenhofer 1967 Armstrong and Hille 1972) associated with a decrease in the current I_P observed after sufficiently long depolarizing test pulses in contradiction to Frankenhaeuser (1962b) this current is now ascribed to an extracellular accumulation of potassium (for references see Bethge et al 1991) It is thus entirely conceivable that the substances studied in these experiments block potassium uptake by the Schwann cell and thereby cause a periaxonal K⁺ accumulation during relatively long test pulses (Landon and Hall 1976, Berthold 1978) The resulting breakdown of the potassium equilibrium potential could then be the cause of the observed K⁺ transients (Bethge et al 1991) This would

mean that the compounds studied here, unlike substances with a time-independent blocking action such as (extracellularly applied) TEA, Flaxedil and 4-AP (Bethge et al. 1991), do not block the potassium channels of the axolemma (Fig. 7, ▲), i.e. the permeability constant \bar{P}_K , but rather the potassium channels of the Schwann-cell membrane (●).

In this case, 5-MOP would occupy a position intermediate between the pure \bar{P}_{K^-} blockers and the blockers that produce K⁺ transients, inasmuch as 5-MOP has a time-dependent action but blocks primarily the permeability constant \bar{P}_K , that is, the potassium channels of the membrane of the node of Ranvier *per se*. If the observed K⁺ transients should indeed prove to be based on enhanced extracellular potassium accumulation, our previous functional model with reference to the potassium-channel blockers we investigated (Bohuslavizki et al. 1992), apart from 5-MOP, would be ruled out. On the other hand, of course, it is possible that our previous hypothesis does not hold also for the action of potassium-channel blockers with a predominantly time-independent action; in this case, it is unlikely that the potassium channel-blockers tested here may selectively block the so-called flickering potassium channel of the axolemma (Koh et al. 1992), which is active in the region of the resting potential. However, the observation that the blockers tested here cause no depolarization at the intact node of Ranvier may be evidence against an increased accumulation of potassium in the periaxonal space (Bethge et al. 1991). A definitive clarification of the site and mechanism of action of the substances we have tested must therefore await experiments with artificially demyelinated axons in which the patch clamp technique (Hamill et al. 1981) is also employed.

Therapeutic considerations. Of the substances tested here, only 5-MOP satisfies our previous hypothesis for the symptomatic therapy of demyelinating diseases with potassium-channel blockers (Bautz et al. 1989; Bohuslavizki et al. 1992). However, according to the alternative hypothesis presented in this paper regarding the mechanism of action of the substances that cause the K⁺ transients (see Fig. 7), such substances could counteract a hyperpolarization resulting from demyelination, by depolarizing the axolemma, and hence could themselves promote normalization of the axolemmal resting potential in the demyelinated region. In this case, the other substances that produce K⁺ transients (see, e.g., Bohuslavizki et al. 1993a,c; 1994) but do not appreciably reduce the potassium permeability constant \bar{P}_K (Fig. 6) could also, in principle, be of therapeutic benefit. This would considerably increase the number of potential medications; however, it is not yet known whether in pathologically demyelinated regions of the axon the Schwann cell can remain sufficiently functional to exert a decisive control over the potassium concentration in the periaxonal space.

For potassium-channel blockers to be used in the treatment of demyelinating diseases, it is essential for the blocking to be sufficiently selective, because any impairment of sodium permeability will inhibit impulse conduction. In this respect,

too, 5-MOP is a particularly suitable medication, the clinical efficacy of which has already been demonstrated (Bohuslavizki et al. 1993a). In addition, 5-acetyl-4-methoxy-6-hydroxybenzofuran is promising from the viewpoint of membrane physiology (Bohuslavizki et al. 1993a). Neither among the acridinons (Bohuslavizki et al. 1993c) nor among the coumarins (Bohuslavizki et al. 1993d, 1994) have we yet discovered highly effective potassium-channel blockers with comparably high selectivity.

Methoxy-substituted psoralens, because of their cutaneous light reaction, have long been administered briefly in combination with UVA radiation (PUVA therapy) to treat psoriasis (Mortazawi and Oberste-Lehn 1973). Demyelinating diseases, in contrast, require long-term therapy. Here the problem of undesirable side-effects becomes obvious. In animals, dosages far above that customary in PUVA therapy (1.5 mg/kg/day; maximally 5 × per week for 10 weeks) were needed to produce symptoms of toxicity (see, e.g., IARC Working Group 1986; Herold and Berbey 1981). In contrast, in the case of MS-related visual field defects, even a single administration of about 5 mg methoxy-substituted psoralens (Bautz 1994) in the form of *Ruta* tea can cause a significant improvement for one to two days (Bohuslavizki et al. 1993b). For long-term treatment with psoralens, however, a daily intake is necessary, because these substances are rapidly metabolized and excreted (Stolk et al. 1981; Bickers and Pathak 1982; Sullivan et al. 1986; Tanew et al. 1988). With such treatment we would expect that beyond the immediate action, observable within a few hours, there would be an additional beneficial long-term effect associated with delaying the disease-induced breakdown of normal electrical activity in the affected axons, comparable to a training effect (Sammeck 1977).

We would also expect psoralen therapy to have a side effect particularly desirable in the case of MS: the antidepressant action of 5-MOP associated with the stimulation of melatonin secretion (Soutre et al. 1990). This is emphasized by observations of many MS patients under long-term treatment with psoralens of plant origin, who have given us spontaneous reports of a remarkable elevation of mood. Whether the psoralens might also achieve significance in the causal therapy of MS, due to their immunosuppressive action under UV light (see, e.g., Laroche et al. 1991), remains to be seen.

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